Emodin enhances ATRA-induced differentiation and induces apoptosis in acute myeloid leukemia cells

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Abstract. Emodin, an extracted natural compound from the root and rhizome of Rheum palmatum L, has been shown to have multiple biological activities including anticancer functions in previous studies. In this study, we investigated the anti-leukemic activity of emodin alone or emodin in the presence all-trans retinoic acid (ATRA) in acute myeloid leukemia (AML) cells and the potential signaling pathway involved. We demonstrated that emodin could significantly enhance the sensitivity to ATRA and present additive differentiation-inducing effects in AML cell line NB4 cells and, especially, in NB4-derived ATRA-resistant MR2 cells. Further study showed that increasing dose of emodin could effectively induce growth inhibition and apoptotic effects in both cell lines as well as in primary leukemic cells from AML patients. Moreover, the apoptotic induction in AML cells was associated with the activation of caspase cascades involving caspase-9, caspase-3, and poly(ADP-ribose) polymerase (PARP) cleavage. In addition, leukemic cell response to emodin stimuli in vitro was observed through the decreased expression levels of Bcl-2 and retinoic acid receptor α (RARα). Importantly, emodin was demonstrated as a new inhibitor of PI3K/Akt in AML cells, even in primary AML cells. It inhibited Akt phosphorylation (p-Akt) at Ser473 as efficiently as mTOR at Ser2448. Consistently, it exerted suppression effects on the phosphorylation of mTOR downstream targets, 4E-BP1 and p70S6K. Taken together, these findings indicate that emodin might be developed as a promising anti-leukemic agent to improve the patient outcome in AML.

Introduction

Acute myeloid leukemia (AML) is characterized by differentiation arrest and inappropriate proliferation and survival of immature myeloid progenitors. In the majority of patients with AML who achieve a complete remission (CR), the leukemia will recur within 3 years after diagnosis. The survival probability at 1 and 5 year is 70 and 46%, respectively, based on the favorable risk evaluation (1). Acute promyelocytic leukemia (APL) is a unique subtype of AML. APL patients achieve CR with all-trans retinoic acid (ATRA) treatment. However, the remission duration is short due to rapid development of retinoid resistance in some APL patients (2). Deregulation of phosphatidylinositol 3-kinase (PI3K)/Akt pathway may contribute to tumorigenesis, metastasis, and resistance to conventional chemotherapy. PI3K/Akt signaling pathway is frequently activated in AML cells, and it has been considered as a new target of therapeutic intervention for AML patients (3-7). Thus, the interruption of PI3K/Akt signaling pathway should be considered when designing anti-AML therapeutic strategies.

Emodin is a natural component extracted from the root and rhizome of Rheum palmatum L. It has multiple biological activities including antimicrobial, antiviral, anti-inflammatory, anti-ulcerogenic, immunosuppressive and chemo-preventive activities (8-10). The investigations of emodin-mediated anticancer effects are in progress. For example, emodin, as a tyrosine kinase inhibitor, suppressed growth of HER-2/neu-overexpressing breast cancer cells in vivo (11). Emodin and docosahexaenoic acid (DHA) potently promoted arsenic trioxide (As2O3) and interferon-α (IFN-α)-induced cell death in HTLV-I infected cells by generation of reactive oxygen species and inhibition of Akt and AP-1 pathways (12). It was also displayed that emodin enhanced the activity of gemcitabine against pancreatic cancer in mice by promoting the mitochondrial-dependent apoptotic pathway (13). Previous studies in our group have shown that emodin may induce apoptosis and reverse multidrug resistance in HL-60 and HL-60/ADR cells (14-16), but the molecular mechanisms have not been completely elucidated. In the present study, we provided the first demonstration of the potential roles and mechanisms of emodin alone or in combination with ATRA in NB4 cells, ATRA-resistant MR2 cells and especially those

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primary leukemic cells from newly diagnosed AML patients. We showed that low dose of emodin could potentially enhance ATRA-induced terminal differentiation in AML cells, especially in ATRA-resistant cells. Emodin could effectively inhibit proliferation and induce apoptosis with a dose- and time-dependent manner in AML cells as well as ATRA-resistant leukemic cells. The critical mechanism underlying these effects is linked to the inhibition of PI3K/Akt signaling pathway in AML cells. These results suggest that emodin or emodin in combination with ATRA may have benefits in AML therapy.

Materials and methods

Chemicals and reagents. Emodin (C_{15}H_{10}O_{5}, MW: 446.35, HPLC-determined purity >98%) was obtained from Nanjing Qingze Medical Technology Co. (Nanjing, China). Emodin and ATRA (Sigma, St. Louis, MO, USA) were reconstituted in 100% dimethyl sulfoxide (DMSO) to 50,000 µM as a stock solution. The stock solutions were maintained at -20°C and further diluted in culture media before use.

Patients and cell lines. ATRA-resistant MR2 cell line and its parental NB4 cell line were obtained from Shanghai Institute of Hematology, Shanghai Ruijing Hospital, China. Cell lines were cultured in RPMI-1640 supplemented with 10% FBS, penicillin (100 IU/ml) and streptomycin (100 µg/ml) based on the supplier's instructions and established procedures. The primary leukemic cells from peripheral blood samples were obtained from 21 newly diagnosed AML patients. Samples had ≥70% blasts in the initial isolation prior to manipulation. Normal controls were recruited from 6 healthy donors. All patient samples were referred to our hospital for cytomorphological and cytogenetic diagnostics, and were diagnosed as AML according to standard French-American-British (FAB) and WHO criteria. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque density-gradient separation. Inform consent was obtained from all patients and healthy donors in accordance with the Declaration of Helsinki, and all manipulations of human specimens were approved by the institutional review board in Fujian Medical University Union Hospital.

Cell proliferation assay. NB4, MR2 and primary AML cells were plated in 96-well plates and treated in triplicates with emodin, ATRA alone or emodin plus ATRA for 48 or 72 h at 37°C. Cells were then incubated with 5 mg/ml MTT (Sigma) for 4 h. The supernatants were removed and cells were pulsed with 100 µl DMSO. The optical density (OD) was measured for 4 h. The supernatants were removed and cells were pulsed with 100 µl DMSO. The optical density (OD) was measured for 12 h. Cells were harvested and washed twice with PBS, and then stained with Annexin V-FITC/PI (Becton-Dickinson, NJ, USA) according to the manufacturer's instructions. The early apoptotic cells were quantified by flow cytometry. To further analyze the cell cycle distribution and the percentages of late apoptotic cells, NB4 and MR2 cells were harvested after 48 h of treatment with emodin. Cell pellets were incubated with the DNA PREP LPR solutions (Beckman Coulter), followed by staining with propidium iodide on ice. Flow cytometry analysis was performed to determine the fraction of cells in G1/G0, S, and G2/M. The cells undergoing apoptosis were obtained from the distinct sub-G1 region of the DNA distribution histograms.

DNA fragmentation assay. NB4, MR2 and primary AML cells were exposed to different concentrations of emodin for 48 h. Cells were harvested by centrifugation. After washed with PBS, DNA fragmentation was analyzed using the manufacturer's procedures (Beyotime, Shanghai, China). In brief, genomic DNA was extracted, then absorbed by a miniprep spin column and eluted with buffer. Electrophoresis for purified DNA was performed in a 1.5% agarose gel at 35 V for 3 h. DNA was visualized by Goldview Nucleic Acid Stain on Gel Image Analysis System (Peiqing, JS-380A, Shanghai, China).

Cell differentiation assay. NB4 and MR2 cells were seeded at a density of 5.0×10⁴/ml in 6-well plates in the presence of either 10 µM emodin, 1.0 µM ATRA alone or 10 µM emodin in combination with 1.0 µM ATRA at 37°C for 96 h. Cells were harvested, washed twice with phosphate-buffered saline (PBS). Cell differentiation effects were determined by morphological examination, CD11b analysis and NBT staining. Cell morphological features were observed by microscopy after Wright-Giemsa staining. Cell surface differentiation antigen CD11b (Biolegend, San Diego, CA, USA) was measured according to the manufacturer's instruction. Data were acquired by flow cytometry (Beckman Coulter FC500, Fullerton, CA, USA). The nitröblue tetrazolium chloride (NBT) reduction assay was performed as previously described (17,18). Total of 200 cells on each slide were counted under light microscope, and the percentages of NBT-positive cells were calculated in each group.

Apoptosis assay. Briefly, 1.0×10⁵/ml of NB4 and MR2 cells in RPMI-1640 medium with 10% fetal bovine serum were plated in 6-well plates. After 12 h of incubation with emodin, cells were harvested and washed with PBS, and then stained with Annexin V-FITC/PI (Becton-Dickinson, NJ, USA) according to the manufacturer's instructions. The early apoptotic cells were quantified by flow cytometry. To further analyze the cell cycle distribution and the percentages of late apoptotic cells, NB4 and MR2 cells were harvested after 48 h of treatment with emodin. Cell pellets were incubated with the DNA PREP LPR solutions (Beckman Coulter), followed by staining with propidium iodide on ice. Flow cytometry analysis was performed to determine the fraction of cells in G1/G0, S, and G2/M. The cells undergoing apoptosis were obtained from the distinct sub-G1 region of the DNA distribution histograms.

Western blotting. NB4, MR2 and primary AML cells were exposed to emodin at varying concentrations and time points. Total protein was extracted and western blotting was performed as described previously (18). Quantification of the band densitometry was performed by Quantity One Version 4.6.2 Software (Bio-Rad, Hercules, CA, USA). Primary antibodies against human caspase-9, caspase-3, poly(ADP-ribose) polymerase (PARP), p-Akt (Ser473), Akt, p-mTOR (Ser2448), mTOR, 4E-BP1 (53H11), p-4E-BP1 (Thr70), p70S6K and p-p70S6K (Thr389) were obtained from Cell Signaling Technology (Beverly, MA, USA). Bcl-2, RARβ and β-actin antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Rapamycin was obtained from Calbiochem EMD Millipore Corp. (Billerica, MA, USA). LY294002 and PD98059 were obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA).

Statistical analysis. Results are presented as mean ± standard deviation, and statistical comparisons of experimental groups were evaluated by Student's t-test. Statistical significance was defined as P<0.05.
Results

Emodin inhibits cell proliferation in NB4 cells, MR2 cells and primary AML cells. MTT assay was used to examine the effects of emodin on ATRA-resistant MR2 cells and the parental NB4 cells. Consistent with the results of our previous study in HL-60 and HL-60/ADR cells, NB4 and MR2 cells were also sensitive to emodin in a dose-dependent manner (Fig. 1A). Emodin inhibited MR2 cell proliferation with an average IC_{50} value of 34.01±2.40 µM, which was similar to the effect on the parental NB4 cells yielding an average IC_{50} of 37.99±2.30 µM. To identify whether similar effects were found in the primary AML cells, 21 samples from AML patients were treated with increasing concentrations of emodin (ranging from 10 to 80 µM) in vitro for 72 h. As shown in Fig. 1B, the results demonstrated that all of the primary AML cells isolated from 21 different subtypes of AML patients including three AML cases unclassified, although variable in degree, were sensitive to emodin with an average IC_{50} value of 31.22±15.58 µM. While less cytotoxicity was found when healthy PBMCs were exposed to the same setting of emodin in vitro. More than 70% cells from the 6 healthy donors remained alive even though administered with emodin as high as 80 µM (Fig. 1C).

Enhancements of the sensitivity of ATRA in NB4 and MR2 cells were also observed by MTT assay. ATRA at 0.2 µM and emodin at 10 µM alone only induced mild proliferation inhibition in NB4 and MR2 cells. However, the combination of 0.2 µM ATRA and 10 µM emodin significantly raised inhibitory rates in NB4 cells and MR2 cells (P<0.05), which were similar to those achieved by 1 µM ATRA treatment in the two cell lines (P>0.05). When increased up the concentration of ATRA to 1 µM in combination with 10 µM of emodin, the average levels of growth inhibition were significantly enhanced from 54.29±13.85 and 26.58±3.57% in 1 µM ATRA mono-treatment group to 85.77±2.89 and 43.95±4.99% in the combination group in NB4 and MR2 cells, respectively, (P<0.05) (Fig. 1D).

Emodin enhances differentiation induction of ATRA in retinoid-responsive NB4 cells as well as in retinoid-resistant MR2 cells. As shown in Fig. 1D, >90% NB4 and MR2 cells remained alive when treated with emodin at a concentration of 10 µM. To identify whether this non-toxic dose of emodin may enhance ATRA-sensitivity in NB4 cells, especially in its ATRA-resistant subclone MR2 cells, we detected the cellular differentiation effects of 10 µM emodin combined with 1.0 µM ATRA in the two cell lines. Compared with the...
untreated and ATRA-treated cells, emodin only-treated cells presented slight differentiation-inducing effects in either NB4 cells or MR2 cells. As shown in Fig. 2A, the combination of emodin and ATRA synergistically induced terminal granulocytic differentiation manifested as smaller cell size, the disappearance of nucleoli, reduced nuclei-cytoplasm ratio, and condensed, distorted and stab form nuclei. Moreover, the acceleration processes of NB4 and MR2 cell differentiation after the combination treatment were further confirmed by CD11b expression analysis and NBT reduction assay. The averaged levels of CD11b-positive cells in co-treatment group were 73.67±3.76% in NB4 cells and 38.29±6.68% in MR2 cells, NBT-positive cells were 89.17±4.01 and 35.50±4.44%, respectively. CD11b and NBT-positive cells in the combination treatment group were significantly higher than those obtained from either emodin or ATRA alone group (P<0.05) (Fig. 2B and C).

**Emodin induces cell apoptosis in NB4 cells, MR2 cells and primary AML cells.** Next, we assessed the role of emodin on the induction of apoptosis in AML cells. The results showed that emodin exerted apoptotic effects in NB4 and MR2 cells dose-dependently, as assessed by Annexin V-FITC/PI staining assay (Fig. 3A). Quantitative evaluation of apoptotic cells was further determined using cell cycle analysis. When compared with the control group, the sub-G1 (apoptotic cell) population was clearly observable in NB4 and MR2 cells treated with 30 µM emodin. The ratio of apoptotic cells was 21.37±0.72 and 26.78±5.19% in NB4 and MR2 cells, respectively (Fig. 3B). Results also revealed a marked G1/G0 and G2/M phase arrest in NB4 and MR2 cells in the presence of emodin. Furthermore, we observed significantly decreased S phase cell population in the two cell lines under the same experimental condition (Fig. 3C and D). DNA fragmentation assay was applied to assess the effect of emodin on the induction of apoptosis in AML cells. Consistently, we found that both of NB4 and MR2 cells succumbed to emodin-induced the formation of DNA fragments at 48-h treatment (Fig. 3E). Moreover, primary AML cells were also sensitive to emodin stimuli in vitro. As shown in Fig. 3F, the apoptotic DNA fragmentation was present in each emodin-treated primary AML sample group, which further confirmed that emodin exhibited apoptotic induction effects in AML cells.

**Emodin leads to activation of the caspase-dependent pathway and the degradation of RARα protein in NB4 and MR2 cells.** The mechanisms underlying emodin-induced apoptosis in AML cells were also analyzed. As shown in Fig. 4A, emodin treatment reduced the expression levels of procaspase-3 and procaspase-9 in NB4 and MR2 cells in a concentration- and time-dependent manner. The cleavage of caspase-3 was induced in both cell lines. Consistently, results also showed that emodin induced cleavage of PARP, one of the important caspase-3 substrates. PARP was activated by cleaved 116-kDa fragment to 85-kDa fragment. The activation modulated by emodin was enhanced with the longer incubation time and higher dose exposure.

Anti-apoptotic protein, Bcl-2, plays an important role during caspase cascade activation and apoptosis induction. PML/RARα oncoprotein was specifically expressed in APL
cells, which inhibits the granulocyte development at the promyelocytic stage of differentiation. Therefore, we further examined the changes of expression levels of Bcl-2 and RARα protein in NB4 and MR2 cells following the treatment with emodin. Spot densitometry quantification analysis revealed a decrease in Bcl-2 expression from 52 to 26% in NB4 cells and from 82 to 22% in MR2 cells, respectively, when administered with increasing doses of emodin at different time points. Although RARα protein was not affected in NB4 cells with the addition of 30 µM emodin for 15 h, decreased RARα ranging from 49 to 37% were observed when longer incubation time and higher dose were applied. Similarly, the presence of emodin also resulted in a dose- and time-dependent degradation (ranging from 69 to 14%) of RARα protein in MR2 cells (Fig. 4A).

**Emodin inhibits activation of the PI3K/Akt signaling pathway in AML cells.** Constitutive PI3K/Akt activation is frequently observed in AML samples and sustains leukemic cell growth (5-7,19,20). Hence, we tested whether emodin may affect PI3K/Akt signaling pathway in AML cells. The results demonstrated that emodin markedly abrogated phosphorylation of Akt at Ser473 and of mTOR at Ser2448 in both NB4 and MR2 cells. Of note, emodin also inhibited mTOR downstream targeted proteins p-4E-BP1 and p-p70S6K in the two cell lines in a dose- and time-dependent manner (Fig. 4A). To further validate our observation that emodin downregulates the activation of crucial molecules in PI3K/Akt pathway, PI3K/Akt inhibitor (LY294002), mTOR inhibitor (rapamycin) and MAPK inhibitor (PD 98059) were administered parallelly with two different concentrations of emodin in NB4 and

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![Figure 3](image-url)
MR2 cells. Compared with the untreated group and the lower dose of emodin (8 µM) treatment group, the inhibitory effects on the phosphorylation of Akt, mTOR, 4E-BP1 and p70S6K induced by 80 µM emodin were as strong as those following by LY294002 and rapamycin treatment. As expected, neither Akt nor mTOR or its downstream target activity in PI3K/Akt pathway were suppressed by PD 98059 in NB4 and MR2 cells (Fig. 4B).
To identify the potential clinical efficacy using emodin as a new PI3K/Akt inhibitor, 21 primary AML samples were included in this study. Eighteen out of the 21 AML samples presented constitutive Akt activation. It is similar to previously reported data that Akt phosphorylation at Ser473 can be detected in 50-80% of AML patients (5,21). Bcl-2 protein could be seen in all of the 21 patient samples as well. The results show that emodin suppressed Akt phosphorylation in a dose-dependent manner among these 18 primary AML samples, which was consistent with the results we observed in NB4 and MR2 cells. Similar downregulation effects on Bcl-2 expression were found in all of the 21 detected samples. The phosphorylation form of mTOR was detectable in 8 out of the 18 AML samples with constitutive Akt activation. Interestingly, the presence of increasing doses of emodin significantly abrogated mTOR activation along with phosphorylated Akt inhibition in these 8 samples. The results from three representative AML samples (patient nos. 8, 13 and 17) are shown in Fig. 5. These strongly indicate that the inhibition of PI3K/Akt activation involved in the anti-leukemia activity is triggered by emodin.

Discussion

AML is a clonal hematopoietic stem cell disorder characterized by differentiation arrest, inappropriate proliferation and survival of immature myeloid progenitors. The prognosis of the disease remains poor, and the mortality rates in AML patients are high despite considerable improvements in therapy. The long-term follow-up studies showed a relapse rate exceeding 20% in high-risk APL patients (22-24), a unique subtype of AML, although the targeted therapy with ATRA and As2O3 was introduced in the clinic. The investigation of novel anti-leukemic agent might generate considerable benefits for AML patients.

The antitumor activities of emodin have been reported. For example, emodin in combination with clinically achievable doses of DHA reduced arsenic concentrations by 100-fold while still remaining highly toxic to tumor cells (12). Emodin may improve the expression of globin genes in K562 cells and also induce K562 cells to erythroid differentiation possibly via upregulating ALAS2 and c-KIT and downregulating miR-221 and miR-222 (25). In this study, emodin exhibited significant anti-leukemic effects in vitro. We provided the first demonstration that nontoxic dose of emodin (< IC_{50}) inhibited growth and induced differentiation to a low degree in NB4 and MR2 cells. However, the administration of low concentration of emodin along with ATRA synergistically enhanced ATRA-induced terminal differentiation in NB4 cells. Especially, the emodin/ATRA combination also significantly increased CD11b-positive cells, NBT-response and differentiation-related morphological features in ATRA-resistant MR2 cells. More interestingly, when increasing dose of emodin was administered individually, we found that it presented proliferation inhibition dose-dependently in the two cell lines as well as in primary leukemic cells from AML patients. We further showed that administration with emodin caused AML cells to undergo apoptosis as evidenced by increasing proportion of Annexin V-FITC-positive cells, sub-G1, cell cycle arrest and obvious DNA fragmentation.

The underlying mechanisms responsible for emodin-induced apoptosis in AML cells were also explored in this study. Muto et al showed that the activation of caspase-3 and -9 can be triggered by emodin in multiple myeloma cells (26). AMAD, an emodin azide methyl anthraquinone derivative, was reported to induce apoptosis in the human breast cancer cell line MDA-MB-453 and the human lung adenocarcinoma Calu-3 cells via a collapse of the mitochondrial membrane potential and the activation of caspase cascades (27). As shown in this study, caspase-3, caspase-9 and PARP precursors were decreased, while the cleavage of caspase-3 and PARP were induced in AML cells in response to emodin. Emodin triggered the activation of caspases in AML cells consistent with the previous findings in multiple myeloma cells, breast cancer cells and lung adenocarcinoma cells (26,27).

Bcl-2 is one of the important anti-apoptotic proteins of the Bcl-2 family, which has been shown to contribute to drug resistance in various human malignancies including hematologic malignancies and solid tumors. Overexpression of
Bcl-2 is associated with poor prognosis (28-31). We observed that emodin suppressed Bcl-2 expression in ATRA-resistant MR2 cells as well as in its parental NB4 cells. Of note, dose-dependent blockage of Bcl-2 expression was further identified in 21 cases of primary AML cell samples when cultured in the presence of emodin. An in vivo study in our group recently demonstrated that consecutive treatment with emodin could effectively downregulate Bcl-2 in AML in a xenograft model in nude mice (data not shown). These indicate suppression of Bcl-2 may contribute apoptosis execution in emodin-treated AML cells.

The promyelocytic leukemia (PML)/RARα fusion protein formed as a result of the (t(15;17)) translocation was found in most of APL patients. As retinoic acid receptors, APL blasts express large amounts of PML/RARα, RARα and RXR isoforms. To activate PML/RARα oncoprotein degradation appears to represent a critical parameter for successful treatment of APL (32-35). Broad networks of post-transcriptional suppressive pathways are activated during ATRA-induced growth inhibition processes in APL cells (36). Interestingly, we found emodin significantly induced loss of RARα protein in the same manner as Bcl-2 protein in NB4, especially in MR2 cells. It remains to be further investigated how emodin exerts degradation effects on RARα protein.

The constitutive activation of Akt pathway plays a critical role in the progression of AML cases. In the present study, we assessed whether the inhibition of PI3K/Akt pathway was concurrently involved in emodin-mediated apoptosis in AML cells. PI3K/Akt signaling pathway is comprised of a family of intracellular protein kinases. Akt activation indirectly promotes transcription of anti-apoptotic genes, while it directly phosphorylates the downstream target, mTOR. Phosphorylated mTOR promotes cell cycle transition from the G1 to S phase via phosphorylation of the downstream targets p70S6K and 4E-BP1, which favor translation of mRNAs for certain growth-promoting proteins such as Cyclin D. We demonstrated that emodin is the direct inhibitor of Akt activation in AML cells. Importantly, it also inhibited mTOR leading to reduced phosphorylation of 4E-BP1 and p70S6K in AML cells. Accordingly, the downregulation of anti-apoptotic protein Bcl-2, cell cycle arrest to G0 phase and reduction of S phase cell population were found in the emodin-treated leukemic cells. Of note, emodin abrogated phosphorylation of Akt at Ser473, which is the site responsible for activating Akt in a feedback loop and has been implicated in rapamycin failure after prolonged treatment (6,7,37,38). Our results indicated emodin may prevent the feedback loop. Moreover, the inhibition of PI3K/Akt pathway by emodin was further confirmed by comparison with PI3K/Akt inhibitor LY294002 and mTOR inhibitor rapamycin, as well as MAPK inhibitor PD98059. Thus, the downregulation of PI3K/Akt pathway is an important molecular mechanism underlying emodin-induced apoptosis in AML cells. Whether there are other pathways, such as ERK1/2 pathway, in response to emodin stimuli in AML cells will be further identified.

In conclusion, we have demonstrated that emodin could enhance ATRA-induced differentiation in APL cells, even in ATRA-resistant leukemic cells. We further showed that emodin induced AML cell apoptosis dose- and time-dependently through inhibition of the PI3K/Akt signaling pathway involving activation of the caspase cascades. To this end, emodin may be considered as a new promising anti-leukemic agent to overcome ATRA-resistance and to improve clinical outcome of AML. Therefore, it would be worthy of extensive experimental investigations in vivo.

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References


